Part #20

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
David J. Kyle	)	
	)	
Serial No. 07/944,739	)	Examiner: Jordan
	)	
Filed: September 14, 1992	)	Group Art Unit: 1205
	)	
For: MICROBIAL OIL MIXTURES AND USES THEREOF	)	

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

I, David J. Kyle, declare as follows:

1. I am a principal scientist and Vice President of Nutritional Products with Martek Biosciences Corporation ("Martek") and am the inventor of the invention described and claimed in U.S. patent application number 07/944,739.

2. I was awarded my Ph.D. in 1981 from the University of Alberta. My doctoral studies concerned plant biochemistry.

3. I began employment with Martek in September 1985 and have continuously been associated with the company since that time.

4. While at Martek, I have engaged in research concerning the production of various triglycerides by different

organisms as well as the modification of triglycerides by various techniques.

5. I have reviewed the outstanding Office Action and the references cited therein, in particular the Long reference (PCT WO 89/00606, 26/01/89) ("Long").

6. After reviewing the Long reference and attempting to follow its teachings, I have concluded that it is non-enabling for the production of a single-cell oil. My conclusion is based upon experimental work performed under my supervision which attempted to practice the examples in Long and upon my review of the reference in light of my personal experience with the cultivation of various organisms in attempts to produce single-cell oils.

7. The practice of Long's teaching was attempted as follows. *C. cohnii* was obtained from various public depositories and cultivated in accordance with the techniques set forth in Long's examples 1-5.

8. The results of these experiments are set forth in Tables I and II.

Table I

Mg DHA/L

Crypthecodinium cohnii strain	Long #1	Long #2	Long #3	Long #4	Long #5
UTEX L1649	0.0	n.g.	1.3	11.9	n.g.
ATCC 30021	n.g.	n.g.	n.g.	n.g.	n.g.
ATCC 30336	n.g.	n.g.	n.g.	n.g.	n.g.
ATCC 30543	n.g.	n.g.	2.3	5.5	n.g.
ATCC 30572	n.g.	20.2	n.g.	n.g.	n.g.
ATCC 50050	n.g.	81.3	n.g.	n.g.	n.g.
ATCC 40750	1.2				

Total fat content of strains that did show growth ranged from 2-10%. Biomass density varied from .6-1.29 g/L. "n.g." = no growth, Long #1 - #5 are 5 permutations of the media described in Long's examples as shown below.

Table II
Major Medium Components

	Instant Ocean	Glucose (g/L)	Yeast Extract (g/L)	Peptone (g/L)
Long #1	100%	1	0.1	0
Long #2	100%	5	0.1	0
Long #3	100%	1	1.0	1.0
Long #4	100%	5	1.0	1.0
Long #5	As per Example 5 of his application			

9. Table I indicates that the vast majority of strains cultivated in accordance with Long's teachings (one of which is that provided by the ATCC) do not exhibit any growth at all. Of those few strains which do exhibit growth, none produced observable quantities of single-cell oil. Most produced fatty acids in such small quantities that it can be concluded that no

oil was produced. Moreover, the only strain to produce any significant DHA, ATCC 50050, under Long #2 may have been erroneously measured. I have attempted to replicate this experiment twice and not succeeded. No growth was obtained with this strain in Long #2. It is my belief that the flasks containing Martek media and Long #2 were mislabeled, which explains the lack of growth reported in Table III.

10. Table III sets forth the results obtained from *C. cohnii* cultivated in accordance with the procedures described in the present application.

Table III

	C. cohnii strain	Martek Growth Conditions mg DHA/liter
UTEX	L1649	37.7
ATCC	30021	ng -
ATCC	30336	31.8
ATCC	30543	44.3
ATCC	30572	146.3
ATCC	50050	ng -
ATCC	40750	540.2

11. As can be seen by comparing Table I with Table III, much greater DHA production was obtained when the organisms were cultivated according to the procedures disclosed in the present application. As stated in paragraph 9, I believe that the flask containing Martek media 50056 was in Martek media, not Long media, and ATCC 50050 was mislabeled and actually contained Long media #5. That would explain the reported no growth. Subsequent attempts to cultivate ATCC 50050 in Martek media also have not proven successful. With respect to both ATCC 50050 and ATCC 30021, it is possible that the samples furnished by the ATCC were not viable and the reported value, in one instance, of 81.3 mg/l was an error.

12. In my opinion, the various media described by Long, which normally are used for fungal cultivation, are inadequate for algal growth and oil production.

13. From the experiments performed under my direction, I conclude that Long would add to the body of prior art cited in the present specification which teaches that successful cultivation of algae such as *C. cohnii* is so difficult as to make this organism unsuitable for production of useable amounts of PUFAs.

14. Because Long does not enable the production from algae such as *C. cohnii* of a single-cell oil, or even recognize or suggest that such an oil exists, Long actually teaches those of ordinary skill in the art that such algae cannot be used to produce a single cell oil containing DHA. In other words, if those of skill in the art attempted to follow the teaching of Long, as was done in my laboratory, they would conclude that *C. cohnii* could not be used to produce oil.

15. Long does not teach the cultivation of algae such as *C. cohnii* in a fermentor and, therefore all experiments were performed in shake flasks. Long would add to the body of knowledge teaching that such algae are shear sensitive, such that they cannot successfully be cultivated in a fermentor.

16. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date	David J. Kyle